Supplementary Materials

Title: Test performance evaluation of SARS-CoV-2 serological assays

Table of Contents

Author Names and Affiliations:
Jeffrey D. Whitman, M.D., M.S.[1]*, Joseph Hiatt, B.A.[2,3,4,5,6,7]*, Cody T. Mowery, B.S.[2,3,5,6,7]*, Brian R. Shy, M.D., Ph.D.[1]*, Ruby Yu, Ph.D.[5,7]*, Tori N. Yamamoto, Ph.D.[5,6,7], Ujjwal Rathore, Ph.D.[4,5,6,7], Gregory M. Goldgof, M.D., Ph.D.[1], Caroline Whitty, B.S.[1,5,7], Jonathan M. Woo, B.S.[5,6,7], Antonia E. Gallman, B.S.[2,5,8], Tyler E. Miller, M.D., Ph.D.[36] , Andrew G. Levine, M.D., Ph.D.[1], David N. Nguyen, M.D., Ph.D.[5,6,9], Sagar P. Bapat, M.D., Ph.D.[1,5,7], Joanna Balcerek, M.D., Ph.D.[1], Sophia A. Bylsma, B.S.[20], Ana M. Lyons, B.S.[21], Stacy Li, B.S.[21], Allison Wai-yi Wong, Ph.D.[2], Eva Mae Gillis-Buck, M.Phil.[10], Zachary B. Steinhart, Ph.D.[5,7], Youjin Lee, Ph.D.[5], Ryan Apathy, B.S.[5,6,7], Mitchell J. Lipke, B.S.[5,7], Jennifer Anne Smith, Ph.D.[7,11], Tina Zheng, B.S.[2,3,12,13], Ian C. Boothby, B.A.[2,14], Erin Isaza, B.S.[2,15], Jackie Chan, B.S.[5], Dante D. Acenas II, B.A.[5], Jinwoo Lee, Ph.D.[2,16], Trisha A. Macrae, Ph.D.[2,16], Than S. Kyaw, B.S.[2,5], David Wu, B.S.[2,3], Dianna L. Ng, M.D.[13,17], Wei Gu, M.D., Ph.D.[1], Vanessa A. York, B.S.[18], Haig Alexander Eskandarian, Ph.D.[18], Perri C. Callaway, B.A.[18,19], Lakshmi Warrier, B.S.[18], Mary E. Moreno, B.S.[18], Justine Levan, Ph.D.[18], Leonel Torres, B.S.[18], Lila A. Farrington, Ph.D.[18], Rita Loudermilk, B.S.[22], Kanishka Koshal, M.P.H.[22], Kelsey C. Zorn, M.H.S.[23], Wilfredo F. Garcia-Beltran, M.D., Ph.D.[36], Diane Yang, Ph.D.[36], Michael G. Astudillo, M.D.[36], Bradley E. Bernstein, M.D., Ph.D.[36], Jeffrey A. Gelfand, M.D.[37], Edward T. Ryan, M.D.[37], Richelle C. Charles, M.D.[37], A. John Iafrate, M.D., Ph.D.[36], Jochen K. Lennerz, M.D., Ph.D.[36], Steve Miller, M.D., Ph.D.[1], Charles Y. Chiu, M.D., Ph.D. [1,9,24], Susan L. Stramer, Ph.D.[25], Michael R. Wilson, M.D. [3,22], Aashish Manglik, M.D., Ph.D.[27], Chun Jimmie Ye, Ph.D.[28,29,30,31,32,33], Nevan J. Krogan, Ph.D.[4,34,35], Mark S. Anderson, M.D., Ph.D.[7], Jason G. Cyster, Ph.D.[5,8], Joel D. Ernst, M.D.[18], Alan H. B. Wu, Ph.D.[1], Kara L. Lynch, Ph.D.[1], Caryn Bern, M.D., M.P.H.[33]**, Patrick D. Hsu, Ph.D.[6,20]**, Alexander Marson, M.D., Ph.D.[4,5,6,7,11,13,28,29,30]**
*Co-first author
**Co-corresponding author

SUPPLEMENTARY METHODS:

Enzyme-Linked Immunosorbent Assays (ELISAs): Epitope Diagnostics assays were performed in singlicate (due to the number of plates available for this study) and carried out according to manufacturer's instructions. Briefly, for IgM detection, 100uL of control samples or 10ul of patient serum and 100ul of sample diluent were added to indicated wells. Plates were incubated for thirty minutes at 37°C and manually washed 5x in provided Wash Buffer. Each well received 100uL of HRP-labeled COVID-19 antigen, was incubated for thirty minutes at 37°C, and was manually washed 5x in provided Wash Buffer. Each well then received 100uL of colorimetric substrate, was incubated for twenty minutes, and then received 100uL of Stop Solution. The absorbance at 450nm (OD450) was measured using a BioTek Synergy H1 Microplate Reader within ten minutes of adding Stop Solution. Positive cutoff for IgM detection were calculated as described in the Epitope Diagnostics protocol: IgM Positive cutoff = 1.1 * ((average of negative control readings) + 0.10). Values less than or equal to the Positive cutoff were interpreted as Negative. For IgG detection, 1uL of serum was diluted 1:100 in Sample Diluent and loaded into designated wells. Plates were incubated for thirty minutes at room temperature and manually washed 5x in provided Wash Buffer. Each well received 100uL of provided HRP-labeled COVID-19 Tracer Antibody, plates were incubated for thirty minutes at room temperature, and manually washed 5x in provided Wash Buffer. Then, each well received 100uL of Substrate, was incubated for twenty minutes, and then received 100uL of Stop Solution. The absorbance at 450nm (OD450) was measured using a BioTek Synergy H1 Microplate Reader within ten minutes of adding Stop Solution. Positive cutoffs for IgG detection were calculated as described in the Epitope Diagnostics protocol: IgG Positive cutoff = 1.1 * ((average of negative control readings) + 0.18). Values less than or equal to the Positive cutoff were interpreted as Negative.

An in-house RBD-based ELISA was performed with minor deviations from a published protocol (Amanat *et al.* 2020, Krammer Lab, MSSM, New York, NY, USA). SARS-CoV-2

Receptor Binding Domain (RBD) protein was produced using the published construct (NR-52306, BEI Resources) by Aashish Manglik (UCSF). 96-well plates (3855, Thermo Scientific) were coated with 2ug/ml RBD protein and stored at 4°C for up to five days before use. Test serum aliquots (12uL), as well as pre-July 2018 historical Negative Control serum from two donors and Positive Control serum from a patient with confirmed anti-SARS-CoV-2 IgG, were diluted 1:5 in 1X PBS (10010-023, Gibco), mixed, and heat inactivated at 56°C for one hour. RBD-treated plates were washed 3x with PBS-Tween (PBST, BP337-500, Fisher Bioreagents) using a BioTek 405 TS Microplate Washer and blocked with PBST-Milk (3% w/v, AB10109-01000, AmericanBio) for one hour at 20°C. Samples were further diluted 1:10 (1:50 final) in PBST-Milk (1% w/v) and 100uL was transferred to the blocked ELISA plates in duplicate plates. Samples were incubated for two hours at 20°C and washed 3x with PBST. The peroxidase AffiniPure Goat Anti-human IgG ($F(ab')_2$ specific) secondary antibody (109-035-097, Lot 146576, Jackson ImmunoResearch) used in this study binds the IgG light chain and has some reactivity for other isotypes (IgM, IgA). This secondary antibody was diluted 1:750 in PBST-Milk (1% w/v), 50ul was added to each sample well, and samples were incubated for one hour at 20°C. Plates were subsequently washed 3x with PBST. We dispensed 100uL of 1x SigmaFast OPD Solution (P9187, Sigma-Aldrich) to each sample well and incubated plates for ten minutes at room temperature. We added 50ul of 3M HCl (A144-212, Fisher Chemical) to stop the reaction and immediately read the optical density at 490nm (OD490) using a BioTek Synergy H1 Microplate Reader. OD490 values were corrected for each plate by subtracting the mean value of each plate's blank wells. To determine a cutoff for positive values, we calculated the mean value of negative wells for each plate, plus three standard deviations.

Assay	Supplier	Product	Antigen *	Format**	Lot(s)	Product Number	Distributor	Kit Acquisition for Study	Performance Notes
LFAs	**BioMedomics** Inc, Morrisville, NC, USA	COVID-19 IgM and IgG Rapid Test	RBD	1	2020032103	51-002-20	Henry Schein, Melville, NY, USA	Provided by Distributor Free of Charge	Some control band inconsistency
	Bioperfectus Technologies Co Ltd, Jiangsu, China	PerfectPOC Novel Corona Virus (SARS-CoV-2) IgM/IgG Rapid Test Kit	NP, SP	1	20200313, 20200313, 20210312	SC30201W	--------	Provided by Supplier Free of Charge	Extra diluent necessary
	Decombio Biotechnology Co Ltd, Beijing, China	Novel Coronavirus (SARS-CoV-2) IgM/IgG Combo Rapid Test-Cassette	--------	1	--------	--------	--------	Provided by Supplier Free of Charge	Some control band inconsistency
	DeepBlue Medical Technology Co Ltd, Anhui, China	COVID-19 (SARS-CoV-2) IgG/IgM Antibody Test Kit (Colloidal Gold)	--------	1	20200305	--------	--------	Donated by John Hering, who purchased from supplier	Extra diluent necessary, Some control band inconsistency
	Innovita Biological Technology Co Ltd, Qian'an, China	Novel Coronavirus (2019-nCoV) Ab Test (Colloidal Gold)	NP, SP	2	20200304	--------	20/20 GeneSystems, Rockville, MD, USA	Purchased from Distributor	Some band smearing
	Premier Biotech, Minneapolis, MN, USA	COVID-19 IgG/IgM Rapid Test Cassette	--------	1	COV20030071	INGM-MC42S	--------	Purchased from Supplier	Some band smearing
	Sure Biotech, New York, NY, USA; Wan Chai, Hong Kong	SARS-CoV-2 IgM/IgG Antibody Rapid Test	NP, SP	1	COV1252003B	VC012103	--------	Provided by Supplier Free of Charge	--------
	UCP Biosciences, San Jose, CA, USA	Coronavirus IgG/IgM Antibody (COVID-19) Test Cassette	--------	1	SMP20200312, SMP20200313	U-CoV-102	--------	Provided by Supplier Free of Charge	Extra diluent necessary
	VivaChek Biotech Co, Hangzhou, China	VivaDiag™ SARS-CoV-2 IgM/IgG Rapid Test (COVID-19 IgM/IgG Rapid Test)	--------	1	E2003002	VID35-08-011	Everest Links Pte Ltd, Singapore	Purchased from Distributor	Some band smearing
	Wondfo Biotech Co Ltd, Guangzhou, China	SARS-CoV-2 Antibody Test (Lateral Flow Method)	--------	3	W19500318	W195	--------	Donated by David Friedberg, who purchased from supplier	Some band smearing
MGH LFAs	**SD Biosensor**, Suwon-si, Gyeonggi-doz, Republic of Korea	STANDARD Q COVID-19 IgM/IgG Duo	NP	2	QCO1020006	Q-NCOV-01D	Henry Schein, Melville, NY, USA	Provided by Distributor Free of Charge	--------
	Biolidics Limited, Mapex, Singapore	2019-nCoV IgG/IgM antibody detection kit	NP, RBD	1	V20200330	CBB-F015016-V	--------	Purchased from Supplier	--------
	Biomedomics Inc, Morrisville, NC, USA	COVID-19 IgM and IgG Rapid Test	RBD	1	2020022702 2020032103	51-002-20	Henry Schein, Melville, NY, USA	Lot 1 provided by Distributor Free of Charge; Lot 2 purchased from Supplier	--------

ELISAs	**Epitope** Diagnostics, San Diego, CA, USA	KT-1033 EDI™ Novel Coronavirus COVID-19 **IgM** ELISA Kit	NP	--	P630C	KT-1032	--------	Purchased from Supplier	--------
	Epitope Diagnostics, San Diego, CA, USA	KT-1032 EDI™ Novel Coronavirus COVID-19 **IgG** ELISA Kit	NP	--	P637U	KT-1033	--------	Purchased from Supplier	--------
	In-House ELISA	Peroxidase AffiniPure Goat Anti-human IgG (F(ab')2 specific) secondary antibody (Jackson ImmunoResearch)	RBD	--	146576	109-035-097	Adapted from Krammer Lab, Icahn School of Medicine at Mt. Sinai, New York, NY, USA	Lab-developed test	--------

***Antigen:**
NP = Nucleocapsid protein
SP = Spike protein
RBD = Receptor binding domain, Spike protein

****LFA Test Cartridge Format:**
1: Single lane, separate IgM and IgG bands
2: Separate IgM and IgG lanes
3: Single lane, single band for both IgM and IgG

Supplementary Table 1. Immunoassay Kit and Manufacturer Information. **Bold** signifies labels used in text and figures.

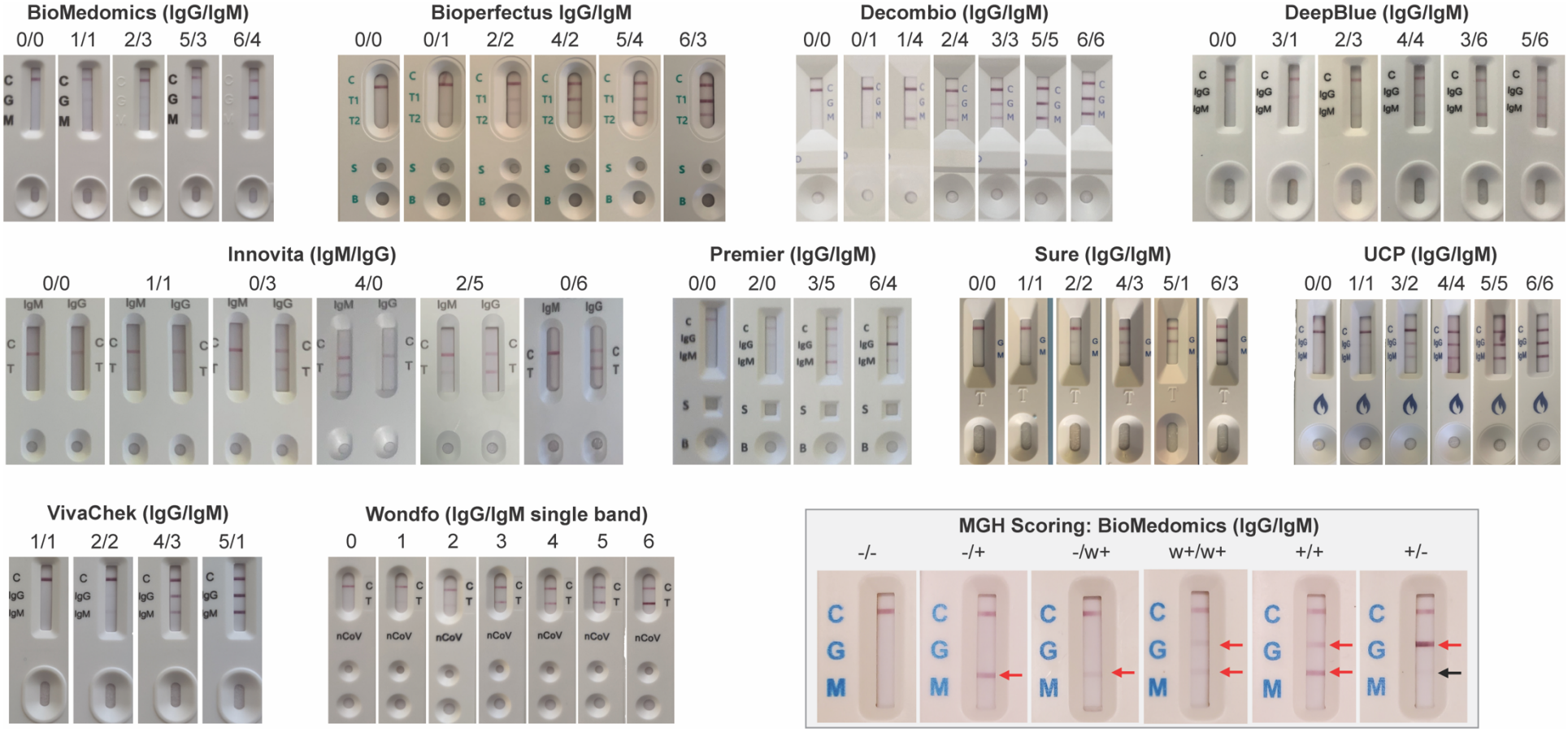
Supplier	IgG			IgM		
	n	Positive Kappa Correlation	Weighted Kappa Correlation	n	Positive Kappa Correlation	Weighted Kappa correlation
BioMedomics	287	0.9651	0.9581	287	0.8247	0.8258
Bioperfectus	277	0.9587	0.9489	277	0.9134	0.8634
DecomBio	285	0.9763	0.9531	285	0.9846	0.9661
DeepBlue	290	0.9549	0.8974	290	0.9218	0.9380
Innovita	252	0.9590	0.8493	251	0.8087	0.8031
Premier	289	0.9719	0.9881	289	0.9681	0.9342
Sure	289	0.9908	0.9666	289	0.9302	0.7971
UCP	289	0.9566	0.9575	289	1.0000	0.9485
VivaChek	269	0.9912	0.9670	269	0.9336	0.9441
Wondfo	273	0.9916	0.9543	-	-	-

Supplementary Table 2. Reader Agreement on Immunochromatographic Lateral Flow Assays (LFAs). Cohen's Kappa correlations were calculated for scores of the IgG band (left) and IgM band (right) of each LFA. The LFA manufactured by Wondfo has a single band for IgG and IgM detection and is displayed here as IgG for convenience. Positive Kappa Correlation: unweighted inter-reader agreement on positive (LFA score > 0) vs. negative (LFA score = 0) reads. Weighted Kappa Correlation: inter-reader agreement on LFA score (0-6), weighted by the square of the difference in reads. All correlations were calculated with the *irr* package version 0.84.1 in R version 3.6.1 using RStudio.

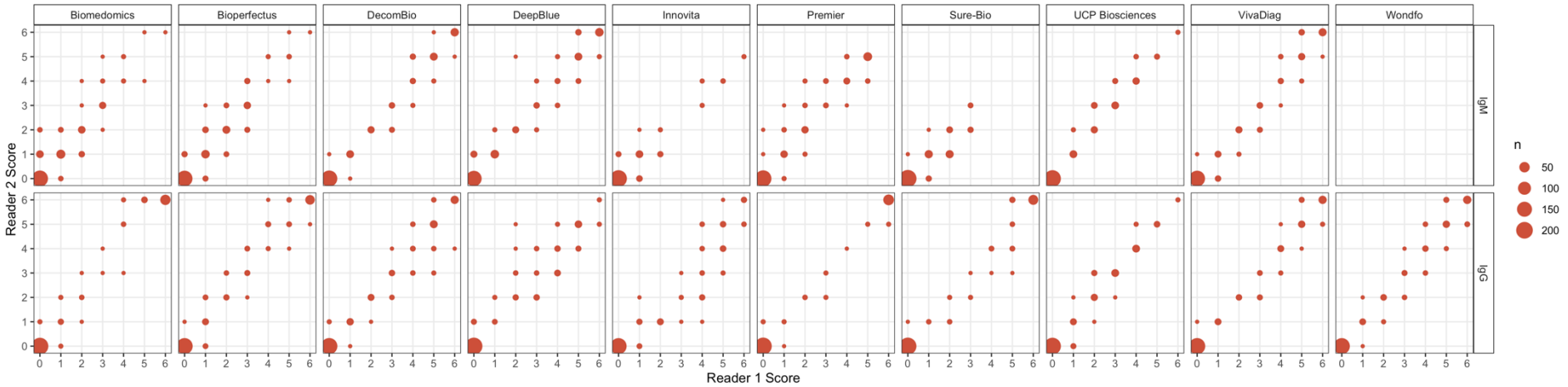
MGH Serology Test Performance Evaluation

	IgM			95% CI		IgG			95% CI		IgM or IgG			95% CI	
Assay	**Total N**	**positive**	**%**	**Lower**	**Upper**	**Total N**	**positive**	**%**	**Lower**	**Upper**	**Total N**	**positive**	**%**	**Lower**	**Upper**
LFAs															
SD Biosensor															
1-5 days	7	0	0.00	0.00	40.96	7	1	14.29	0.36	57.87	7	1	14.29	0.36	57.87
6-10 days	15	6	40.00	16.34	67.71	15	5	33.33	11.82	61.62	15	7	46.67	21.27	73.41
11-15 days	19	15	78.95	54.43	93.95	19	16	84.21	60.42	96.62	19	17	89.47	66.86	98.70
>16 days	7	6	85.71	42.13	99.64	7	6	85.71	42.13	99.64	7	6	85.71	42.13	99.64
Pre-COVID-19	60	0				60	1				60	1			
Biolidics															
1-5 days	7	0	0.00	0.00	40.96	7	0	0.00	0.00	40.96	7	0	0.00	0.00	40.96
6-10 days	15	2	13.33	1.66	40.46	15	8	53.33	26.59	78.73	15	8	53.33	26.59	78.73
11-15 days	19	9	47.37	24.45	71.14	19	16	84.21	60.42	96.62	19	16	84.21	60.42	96.62
>16 days	7	4	57.14	18.41	90.10	7	6	85.71	42.13	99.64	7	6	85.71	42.13	99.64
Pre-COVID-19	60	0				60	0				60	0			
BioMedomics															
1-5 days	7	1	14.29	0.36	57.87	7	0	0.00	0.00	40.96	7	1	14.29	0.36	57.87
6-10 days	15	6	40.00	16.34	67.71	15	6	40.00	16.34	67.71	15	7	46.67	21.27	73.41
11-15 days	19	14	73.68	48.80	90.85	19	14	73.68	48.80	90.85	19	15	78.95	54.43	93.95
>16 days	7	6	85.71	42.13	99.64	7	6	85.71	42.13	99.64	7	6	85.71	42.13	99.64
Pre-COVID-19	60	0				60	0				60	0			

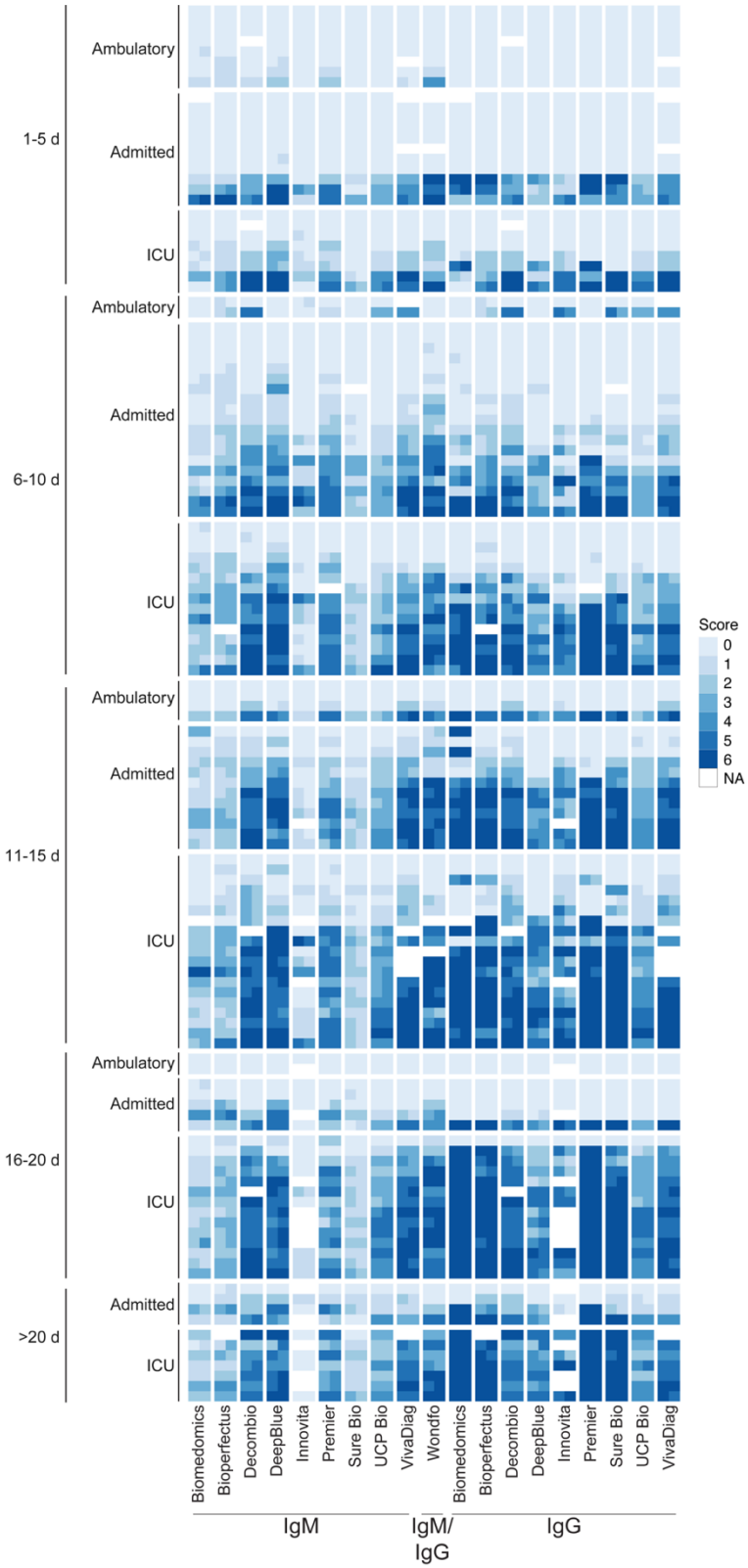
Supplementary Table 3. Assay performance on validation cohort performed at MGH using positivity thresholds based on concordance studies to an MGH-group in-house ELISA. Comparison of MGH and UCSF percent positivity at different positivity thresholds is performed in Supplementary Figure 4. Note, the one negative patient included in the >16-day timepoint was immunocompromised.



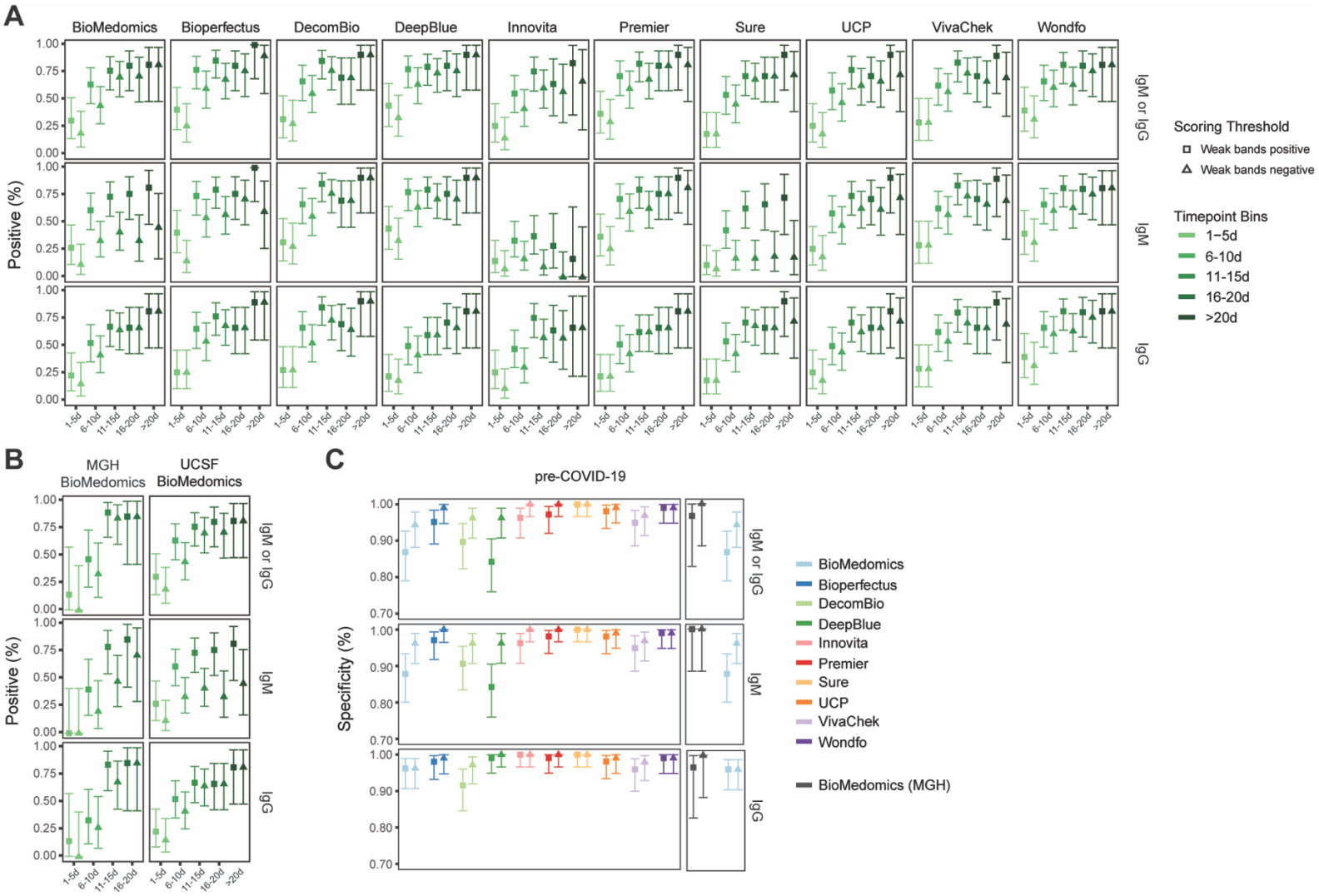
Supplementary Figure 1: Representative images of LFA scoring.



Supplementary Figure 2: Comparison of Reader 1 and Reader 2 LFA scores. The size of each point signifies the number of tests with the indicated reader 1-to-reader 2 score combination. The LFA manufactured by Wondfo has a single band for IgG and IgM detection and is displayed here as IgG for convenience.



Supplementary Figure 3: LFA scores by serological assay according to highest-level clinical care received by the patient by the patient.



Supplementary Figure 4. Comparison of the effect of different positivity thresholds on percent positivity and specificity. **A.** The percent positivity of each assay tested on serum from SARS-CoV-2 RT-PCR-positive patients is plotted by time after patient-reported symptom onset. Squares indicate

percent positivity using Reader Score > 0 (“Weak bands positive”) as the positivity threshold. Triangles indicate percent positivity using Reader Score > 1 (“Weak bands negative”) as the positivity threshold. “IgM or IgG” signifies detection of either isotype. Wondfo reports a single band for IgM and IgG together, and the results are plotted here as both “IgM” and “IgG” for horizontal comparison across assays. **B.** Comparison of percent positivity at each timepoint for BioMedomics assay at either the MGH (left) or UCSF (right) study site using low (square) or high (triangle) positivity thresholds. Note that a weak score at MGH is not directly equivalent to a 1 at UCSF due to difference in reader training. **C.** The specificity of all assays on historical pre-COVID-19 serum using low (square) or high (triangle) positivity thresholds. UCSF BioMedomics data is plotted again in the right subpanel column for direct comparison to MGH BioMedomics data. All error bars indicate 95% confidence intervals.